UNIVERSITY OF GONDAR

FACULTY OF VETERINARY MEDICINE

PREVALENCE OF BOVINE FASCIOLOSIS IN ANIMALS SLAUGHTERED AT SENDAFA-BEKE MUNICIPAL ABATTOIR, OROMIA SPECIAL ZONE SURROUNDING FINFINNE, ETHIOPIA.

DVM THESIS

BY

TAYE DEGU

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GONDAR, ETHIOPIA

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A thesis submitted to Faculty of Veterinary Medicine, University of Gondar in partial fulfillment of requirements for the degree of Doctor of Veterinary Medicine

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BY

TAYE DEGU

Board of external examiners	**signature**
1. Prof. Abebaw Gashaw	
School of Vet .Med, Jimma University	________________
2. Prof. Tadelle Tolla	
School of Vet .Med, Jimma University	________________
3. Dr. Gelagay Ayelet (Assoc. Prof.)	
National Veterinary Inistitute (NVI), Ethiopia	________________
4. Dr. Fufa Dawo (Assoc. Prof.)	
FVM, Addis Ababa University	________________
5. Dr. Ahimed Yassin (Assoc. Prof.)	
FVM, Wollo University	________________
6. Dr. Dessie Shiferaw (Assoc. Prof.)	
FVM, Hawassa University	________________

Thesis advisor:

1. Dr. Reta Tesfaye (DVM, Msc, Assistant professor) ________________

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CDC	Centers for Disease Control and Prevention
E	East
ELISA	Enzyme linked Immunosorbent Assay
FAO	Food and Agricultural Organization
ILRI	International Livestock Research Institute
masl	meters above sea level
MUDC	Ministry of Urban Development and Construction
N	North
Spp	Species
SPSS	Statistical package for social science
WHO	World Health Organization

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ABSTRACT

A cross-sectional study aimed at estimating the prevalence and identifying *Fasciola* species in cattle slaughtered at Sendafa-Beke municipal abattoir was conducted from November, 2014 to April, 2015. The study was based on post-mortem inspection of livers and coprological examination using sedimentation technique. The sensitivity and specificity of sedimentation technique to diagnose *Fasciola* infection as compared to post-mortem inspection of liver was also determined. Out of 384 livers inspected, 134 (34.89%) were positive for *Fasciola* species. *F. hepatica* was found to be the most prevalent species 75 (55.97%) as compared to *F. gigantica* 24 (17.91%). Mixed infection with both species was observed in 12 (8.96%) animals and 23 (17.16%) cattle were infected with unidentified immature liver flukes. Likewise, out of 384 fecal samples examined 83 (21.6%) were positive for *Fasciola* eggs. On assessment for potential risk factors, age and origin, didn't show significant association with the prevalence of infections ($P>0.05$). However, body condition revealed significant disparity ($P<0.05$) as greater magnitude of infections were detected in poor body condition of animals than medium and good body conditions. Besides, the sensitivity and specificity of the sedimentation technique as compared to postmortem finding was 61.9% and 100% respectively. It is concluded that fasciolosis, due to *F. hepatica* and *F. gigantica*, is prevalent in cattle in the study areas. Therefore, it is recommended that strategic control and prevention of the parasite should be implemented and further study on live animals of different age, species and breeds should be conducted. Furthermore, upon diagnosis of faciolosis epidemiological information about the disease with suggestive clinical examination should be considered even in the absence of *Fasciola* eggs during coprological examinations.

Key words: Abattoir, Bovine, Coprology, Fasciolosis, Prevalence, Post- motem, Sendafa-Beke, Sensitivity, Specificity.

1. INTRODUCTION

Ethiopia owns huge number of ruminants having high contribution for meat consumption and generates cash income from export of live animals, meat, edible organs and skin. In spite of the presence of huge ruminant population, Ethiopia fails to optimally exploit these resources due to a number of factors such as recurrent drought, infrastructures problem, rampant animal diseases, poor nutrition, poor husbandry practices, shortage of trained man power and lack of government policies for disease prevention and control (ILRI, 2009).

Among the animal diseases that hinder the animal health, parasitic infections have a great economic impact, especially in developing countries. Out of these parasitic problems of farm animals, fasciolosis is a major disease, which imposes direct and indirect economic impact on livestock production, particularly of sheep and cattle (Keyyu *et al*., 2005; Menkir *et al*., 2007). *Fasciola hepatica* and *Fasciola gigantica* are the two liver flukes commonly reported to cause fasciolosis in ruminants (Walker *et al*., 2008). The infection is acquired through grazing on swampy pasture (Urquhart *et al*., 1996). Acute fasciolosis often remains undetected in cattle and develops to the chronic form which makes them less resistant to other liver infections (Radostits *et al.*, 2007). Chronically infected cattle can show signs such as loss of condition, lethargy, anaemia, bottle jaw, sub-optimal growth rates, diarrhea, metabolic disease and reduced milk yield in dairy cows, and reduced fertility. Signs are exacerbated by poor nutrition or gastro-intestinal parasitism (Mason, 2004; Radostits *et al.*, 2007).

Fasciola hepatica has a cosmopolitan distribution, mainly in temperate zones, while *Fasciola gigantica* is found in tropical regions of Africa and Asia. Thus, the two fasciolid species overlap in many African and Asian countries and sometimes in the same country, although in such cases the ecological requirements of the flukes and their snail intermediate host are distinct (Mas-Coma *et al*., 2005). In developed counties, the incidence of *F. hepatica* can reach up to 77%. In tropical countries, fasciolosis is considered the single most important helminth infection of cattle, with reported prevalence of 30-90% (Spithill *et al.,* 1999). The prevalence of fasciolosis in many parts of Africa has been determined mainly at slaughter, however, estimation of economic loss due to fasciolosis at national or regional level is limited by lack of accurate estimation of the prevalence of disease (Phiri *et al.*, 2005).

Diagnosis of faciolosis is based primarily on clinical signs, seasonal occurrence, prevailing with the patterns and a previous history of fasciolosis on the farm or the identification of snail habitats (Taylor *et al.*, 2007). Confirmatory diagnosis however, is based on demonstration of *Fasciola* egg through standard examination of faeces in a laboratory and post-mortem examination of infected animals and demonstration of immature and mature flukes in the liver (Soulsby, 1982). Immunological tests are also available for demonstration of *Fasciola* infection (Charles *et al*, 2010).

The presence of fasciolosis due to *F. hepatica* and *F. gigantica* in Ethiopia has long been known and its prevalence and economic significance has been reported by several workers (Goll and Scott, 1978; Graber, 1978; Gemechu and Mamo, 1979; Yilma and Malone, 1998; Yilma and Mesfin , 2000; Tolosa and Tigre, 2007; Abunna *et al.,* 2009). In Ethiopia, *Fasciola gigantica* is found at altitudes below 1800 meters above sea level, while *Fasciola hepatica* is found at altitude of 1200-2560 meters above sea level. Mixed infections by both species can be encountered at 1200-1800 meters above sea level (masl) (Yilma and Malone, 1998).

A review of available literature strongly suggests that fasciolosis exists in almost all parts of the country. It is regarded as one of the major setbacks to livestock productivity incurring huge direct and indirect losses in the country. Sendafa-Beke is one of the areas where the environmental conditions and altitude is conducive for the occurrence of fasciolosis. However, no information is available about its prevalence and fasciola species found in the study area. Therefore, the objective of this study was to determine the prevalence of bovine fasciolosis and to identify fasciola species found in Sendafa-Beke municipal abattoir. The sensitivity and specificity of sedimentation technique to diagnose *Fasciola* infection as compared to post-mortem inspection of liver was also determined.

2. LITERATURE REVIEW

2.1. Definition

Fasciolosis is a disease of sheep, goat, and cattle. It occasionally affects humans, hence considered as a zoonotic disease (WHO, 1995; Andrews, 1999). Fasciolosis is also known as, fasciolasis, distomatosis and liver rot (Andrews, 1999).

2.2. Etiology

The disease is caused by digenean trematodes of the genus *Fasciola*, commonly referred to as liver flukes (Urquhart *et al., 1996).* According to Soulsby (1982) and Urquhart *et al*., (1996), the taxonomic classification of the organisms that cause fasciolosis is presented as follows: Phylum: Platyhelminthes, Class: Trematoda, Sub class: Digenea,Super family: Fascioloidea, Genus: *Fasciola,* Species: *F.hepatica* and *F.gigantica*

The two species most commonly implicated as etiological agents of fasciolosis are *F. hepatica* and *F. gigantica*. *F. hepatica* has a worldwide distribution but predominates in temperate zones while *F. gigantica* is found on most continents, primarily in tropical regions (Andrews, 1999). Fasciola hepatica is the most common and important liver fluke and has a cosmopolitan distribution in cooler climates. Lymnaea mud snails are intermediate hosts and release the infective form, the metacercaria, onto herbage. Hepatic fasciolosis is of economic importance in sheep and cattle. *F. hepatica* may infest all domestic animals, including equine and many wild life spp, and sheep are the most important source of pasture contamination. Human cases are usually associated with the ingestion of marsh plants such as water cress. A similar but larger fluke, *F.gigantica*, is restricted to warmer regions including part of Africa and Asia (Radostits *et al.*, 2007).

2.3. Morphology

2.3.1. *Fasciola hepatica*

Gross

The young flukes have lancet like in appearance at the time of entry into liver and are 1-2 mm in length. When they are fully matured in the bile duct they are leaf shaped, grey-brown in color and measure around 2.5- 3.5 cm in length and l.0 cm in width. The anterior end is conical and marked off by distinct shoulders from the body (Urquhart *et al., 1996;* Taylor *et al.*, 2007).

Microscopic

The integument is covered with backwardly projecting spines. An oral and ventral sucker may be readily seen. The egg is thin-shelled, oval, operculate, bown-yellow and large (130-150 μm × 65-90 μm), and about twice the size of a trichostrongyle egg (Taylor *et al.*, 2007).

2.3.2. *Fasciola gigantica*

Gross

The adult fluke is larger than *F.hepatica*, the body is more transparent, and can reach 7.5 cm in length and 1.5 cm in breadth. The shape is more leaf-like, the conical anterior is very short and the shoulders, characteristics of *F.hepatica*, are barely perceptible. (Urquhart *et al., 1996;* Taylor *et al.*, 2007)

Microscopic

The egg is larger than those of *F.hepatic*a, measuring 170-190 × 90-100 μm (Taylor *et al.*, 2007).

A.   B

A). *Fasciola hepatica* (left) and *Fasciola gigantic* (right)   B) Fasciola egg

Figure 1: Adult stage of fasciola spp and their egg (Source: Taylor, 2007)

2.4. Life Cycles

Adult *F.hepatica* flukes live in the bile ducts of ruminant and other mammalian hosts (Taylor *et al.*, 2007). Their eggs are carried first to the bowel lumen with the bile and then to the exterior with the feaces. When deposited, each of these eggs consists of a fertilized ovum and a cluster of vitelline cells enclosed in an operculated capsule. The miracidium is completely covered with cilia and has a conical papilla at its anterior end for boring into the snail intermediate host. The miracidium, which is fully developed hatch out after 2 to 4 weeks at summer temperature, escape from the egg capsule by pushing aside the operculum and swims about in search of a suitable spp of snail. If it fails to find a snail within 24 hours, the miracidium exhausts its energy stores and dies. If the miracidium is more fortunate, it bores in to the snail's body, loses its ciliated covering, migrates to the gonad or digestive gland (often referred to as the liver), and forms a sporocyst. Each germinal cell, by growth and repeated divisions, becomes a germinal ball, and each germinal ball develops into a redia. The redia grow until they brust the sporocyst wall and are thus liberated in to the tissue of the snail. The radia has a mouth and digestive organs eats way through the snail tissues. Like the sporocyst, the radia packed with germinal balls, these being the progenitors of a second generation of radiae. Each germinal ball of the second generation radiae develops in to yet a third kind of larva, the cercaria (Bowman, *et al.*, 2003).

Cercaria attaches to herbage and transform into metacercaria by secreting a tough protective cyst wall. After ingestion by final host, each metacercaria releases an immature fluke which crosses the intestinal wall and migrate across the peritoneal cavity to the liver. The migration is sometimes misdirected and ectopic flukes can be found in the lungs, particularly in cattle. The young *F.hepatica* migrates through the hepatic parenchyma for about 4-5 weeks, growing from 0.1 to 10 mm. After entering the bile duct, they grow more than double their size before egg laying starts at about 10-12 weeks after infestation. Adult cattle may remain carriers for many years because of the longevity of the adult flukes (Radostits *et al.*, 2007). The life cycle of *Faciola* species is depicted in figure 2.

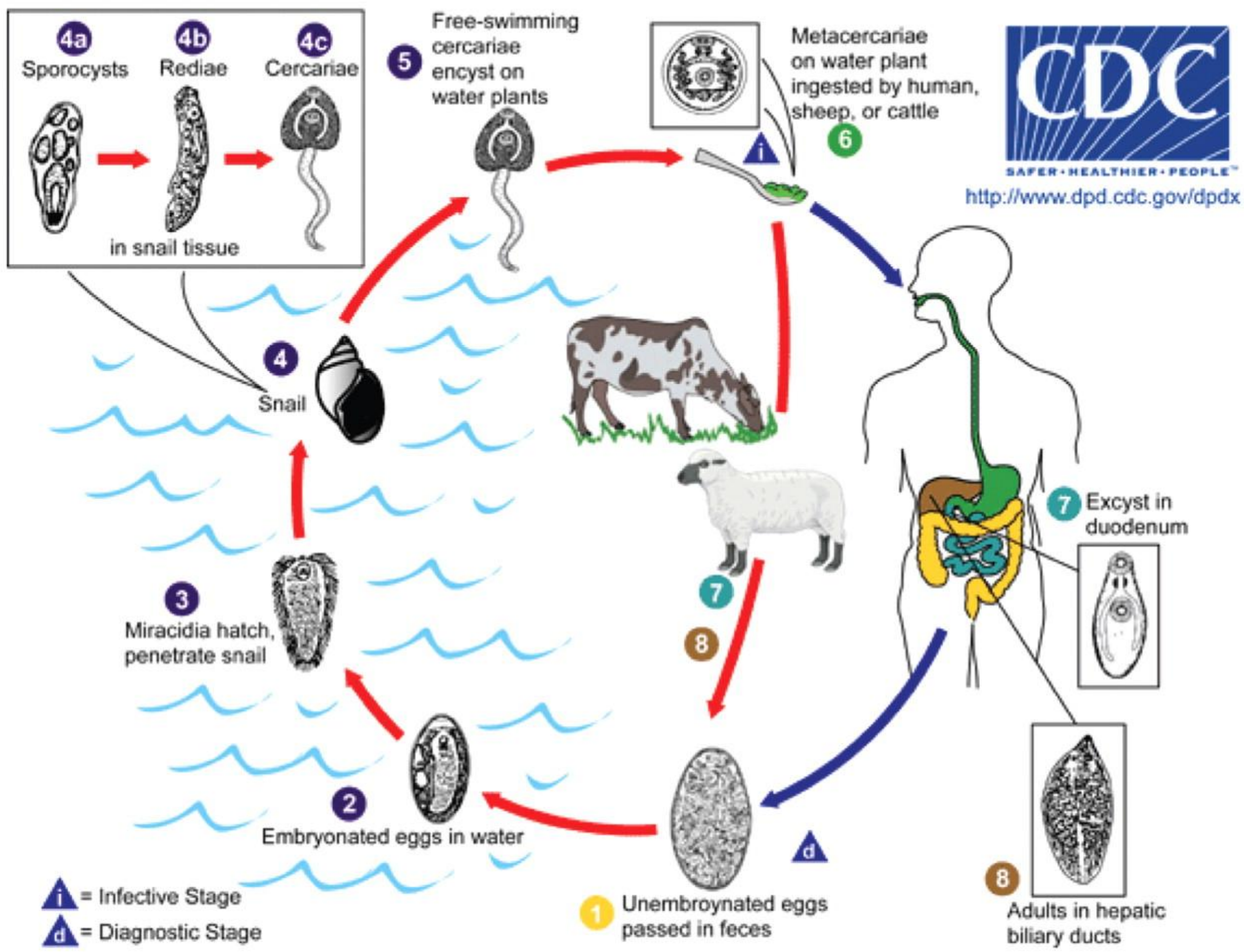

Figure 2 : Life cycle of *Fasciola* (Source: CDC, 2015)

2.5. Epidemiology

The two most important species are *Fasciola hepatica* found in temperate area and in cooler areas of high altitude in the tropics and subtropics and *Fasciola gigantica*, which predominates in tropical area (Biniam *et al*., 2012). In Ethiopia, *F. hepatica* and *F.gigantica* infections occur in areas above 1800 m and below 1200 m above sea level, respectively which has been attributed to variations in the climatic and ecological conditions such as rainfall, altitude, and temperature and livestock management system. In between these altitude limits, both species coexists where ecology is conductive for both snail hosts, and mixed infections prevail (Yilma and Malones, 1998).

The risk of hepatic fasciolosis is determined by the numbers of infected Lymnaea snails in the grazing area. The disease has a predictable seasonal pattern in regions where snails are active for only part of the year. Some Lymnaea snails have a more aquatic habitat than others but all are restricted to damp or wet environments. In general, they prefer non- acidic, low-lying, swampy areas with slowly moving water, but land with small streams, springs, blocked drainage or spillage from water troughs may also be potentially hazardous for grazing stock. Snails burrow in to the soil to survive dry periods and release cercariae when free water is present. Snail habitats may be permanent or temporary. The latter expand and contract depending on water availability. Construction works, such as road building may alter drainage patterns and disease risk. Improvement of peaty pastures by lime application may increase risk by reducing soil acidity and allowing snail colonization (Radostits *et al, 2007*).

2.5.1. Factors that Affect the Distribution of Fasciolosis

The main factors determining the timing and severity of hepatic fasciolosis are those that influence the number of metacercariae accumulating on herbage (Radostits *et al, 2007*). There are three main factors influencing the production of large number of metacercaria necessary for outbreak of fasciolosis.

I. Availability of Suitable Snail Habitats

L.truncatala prefers wet mud to free water, and permanent habitats include the bank ditches or streams and the edges of small ponds. Following heavy rain fall or flooding, temporary habitats may be provided by hoof marks, wheel ruts or rain ponds. Field with clump of rush is often suspect site. Though a slightly acid pH environment is optimal for *L.truncatala*, excessively acid pH levels are determent, such as occur in peat bogs, and area of sphagnum moss. (Urquhart, *et al.,* 1996; Taylor *et al.*, 2007)

II. Temprature

A mean day/night temperature of $10^{0}{}_{C}$ or above is necessary both for snails to breed and for the development *of F.hepatica* within the snail, and all activity ceases at $5^{0}{}_{C}$. This is also the minimum range for the development and hatching of *F.hepatica* eggs. However, it is only when the temperature rises to 15^{0}c and are maintained above that level, that a significant multiplication of snails and fluke larval stages ensues (Urquhart, *et al.*, 1996).

III. Moisture

The ideal moisture conditions for snail breeding and the development of *F.hepatica* with in snails are provided when rain fall exceeds transpiration, and field saturation is attained. Such conditions are also essential for the development of fluke eggs, for miracidia searching for snail and for the dispersal of cercariae being shed from snails (Urquhart, *et al.*, 1996).

2.6. Host Range

2.6.1. Definitive Host

Definitive hosts include cattle, sheep, many other ruminants, equidae, swine and rabbits. Finally it should be remembered that *F. hepatica* can infect wide range of mammals and is possible that on occasions these hosts may act as reservoirs of infection, but *F. gigantica* infect ruminants (Urquhart *et a*l., 1996).

2.6.2. Intermediate Host

The intermediate host for *F.hepatica* includes *Lymnaea truncatula* in the United Kingdom and Europe and *Gelb bulimoides* and others in the United States. *L.columella* has been identified as an intermediate host in Canada and more recently in Brazil. In Newzealand, *L.tomentosa* and *L.*

truncatula have occurred without Fasciolosis becoming a major problem, but the introduction of *L.columella* markedly increased the range and severity of the disease. *L.comentosa* is the major host snail in Australia, although *L.columella* has been reported to be present in non-farming areas and *L.viridis* has also been found (Radostits *et al, 2007*).

For *F.gigantica* the intermediate hosts include snail of the genus *Lymnaea*; in the southern Europe it is *L.auricularia*, which is also the important spp in the southern USA, the Middle East and the Pacific Islands. Other important lymnaea vectors of *F.gigantica* are *L.natalensis* in Africa, *L.rafescens* in Indian subcontinent, *L. rubiginosa* in Southeast Asia and *L. viridis* in China and Japan. All these snails are primarily aquatic snails and are found in streams, irrigation channels and marshy swamps (Taylor *et al.*, 2007).

2.7. Pathogenesis

This varies according to the number of metacercaria ingested, the phase of parasitic development in the liver and the species of the host involved. Essentially the pathogenesis is two-fold. The first phase occurs during migration in the liver parenchyma and is associated with liver damage and hemorrhage. The second occurs when the parasite is in the bile ducts, and results the hematophagic activity of the adult flukes and from damage to the biliary mucosa by their cuticular spine (Taylor, *et al*., 2007).

Fasciolosis may be acute, sub-acute or chronic. The acute disease occur 2-6 weeks after the ingestion of large numbers of metacercaria, usually over 2000, and is due to the sever haemorrhage which results when the young flukes, migrating in to the liver parenchyma, rapture blood vessels. Damage to the liver parenchyma is also severe. At necropsy the liver is enlarged, haemorragic and honey combed with the tract of migrating flukes. Outbreak of acute fasciolosis is generally presented as sudden deaths during autumn and early winter (Urquhart *et al.*, 1996).
In the sub acute disease, metacercaria are ingested over a longer period and while some have reached the bile ducts, where they cause cholangitis, others are still migrating causing lesions less severe, but similar to those of the acute disease; thus the liver is enlarged with numerous necrotic or haemorrhagic tracts visible on the surface and in the substance. Subcapsular haemorrhages are usually evident, but rupture of these is rare. This form of the disease, occurring 6-10 weeks after

ingestion of approximately 500-1500 metacercaria, also appears in the late autumn and winter. Hemorrhagic anaemia and hypoalbuminaemia are the consistent laboratory findings and if untreated, can result in high mortality rate. However it is not as rapidly fatal as the acute condition. The affected animal show clinical signs for 1-2 weeks prior to death; these include a rapid loss of condition, a marked pallor of the mucous membranes, and an enlarged and palpable liver. Sub mandibular or facial oedema and ascites may be present (Urquhart *et al.,* 1996).

Chronic fasciolosis develops only after the adult flukes establish in the bile ducts. Here they cause cholangitis, biliary obstruction, fibrosis, and a leakage of plasma protein across the epithelium. Although this protein can be re-absorbed in the intestine; there is poor utilization and retention of nitrogen leading to hypoalbuminemia. There is also a loss of whole blood due to the feeding activities of the fluke. This exacerbates the hypoalbuminemia and eventually gives rise to anemia (Radostits *et al.*, 2007).

2.8. Clinical sign

Infected cattle can exhibit poor weight gain and dairy cattle have lower milk yield, and possibly metabolic diseases (Mason, 2004). In heavy infection in cattle, where anaemia and hypoalbuminaemia are severe, sub mandibular edema frequently occurs. With smaller fluke burdens, the clinical effect is minimal and the loss of productivity is difficult to differentiate from inadequate nutrition. It must be emphasized that diarrhea is not a feature of bovine fasciolosis unless it is complicated by the presence of ostertagia spp. Combined infection with these two parasites have been referred to as the fasciolosis/ostertagiosis complex (Taylor, *et al.,* 2007).

Fasciolosis ranges in severity from a devastating disease in a sheep to an asymptomatic infection in cattle (Kahn *et al.,* 2005). Acute fasciolosis in sheep most often occurs as sudden death without other apparent clinical abnormality. It is usually seen in the summer and autumn, but may occur at any time when sheep have the opportunity to graze heavily contaminated herbage. The disease is manifested by: dullness, weakness, lack of appetite, pallor and edema of mucosae and conjunctivae and pain when pressure is exerted over the area of the liver. Death occurs quickly and may be accompanied by the passage of blood-stained discharge from the nostrils and anus. Most deaths occur within a period of 2-3 weeks. Acute fasciolosis are rarely occurrs in cattle. Acute and chronic fasciolosis are opposite end of the clinical spectrum. Intermediate forms occur and a sub acute syndrome has been described in sheep. The major clinical signs are weight loss and pallor of the mucous membranes. Submandibular edema will be seen in only a few cases, but many animals will resent palpation over the region of the liver. Chronic fasciolosis does not become apparent until several weeks after the danger of acute disease has receded. Affected sheep lose weight, develop submandibular edema, and pallor of the mucosae over a period of weeks. Cattle also lose weight, especially if lactating, milk production falls and anemia may develop (Radostits *et al.*, 2007).

2.9. Diagnosis

This based on primarily on clinical sign, seasonal occurrence, prevailing weather patterns, and a previous history of fasciolosis on the farm or the identification of snail habitats. While the diagnosis of ovine fasciolosis should present few problems, especially when the post mortem examination is possible, diagnosis of bovine can sometimes prove difficult. In this context, routine haematological tests and examination of faeces for fluke egg (note: eggs of fasciola are brawny yellow and eggs of paramphistomidae are colorless) are useful and may be supplemented by two other laboratory test (Taylor *et al.*, 2007). The first is the estimation of plasma level of enzymes released by damaged liver cells. Two enzymes are usually measured. Glutamate dehydrogenase is released when the parenchymal cells are damaged and levels become elevated with in the first few weeks of infection. The other, gamma glutamyl transpeptidase indicate damage to the epithelial cells lining the bile duct. Elevation of this enzyme takes place mainly after the flukes reach the bile ducts and raised levels are maintained for a longer period. The second is detection of antibodies against components of flukes, ELISA and the passive haemagglutination test being the most reliable (Urquhart *et al.*, 1996; Radostits *et al.*, 2007).

The most direct and reliable techniques for diagnosis of the fasciolosis is liver examination at slaughter or necropsy. In acute fasciolosis, there may be peritonitis, particularly on the visceral surface of the hepatic capsule. Due to migration of flukes, there are dark hemorrhagic streaks and foci. The liver is swollen, friable and has capsular perforations marked by hemorrhagic tags. Calcifications of bile ducts and enlargement of gall bladder is characterstic lesions observed in chronic fasciolosis. Progressive biliary cirrhosis which ultimately produces hard fibrotic liver in which bile ducts are prominent, thickened, and fibrous in cattle (Radostits *et al.*, 2007).

2.10. Treatment

Affected cattle are treated by administration of a fasciolocide. Of those currently used for cattle the commonest are triclabendazole, oxyclozinide, rafoxanide, nitroxynil, albendazole and clorslon. All will remove more than 90 percent of adult flukes from the ducts, but they have variable efficiencies against the immature stages migrating through the liver. The most effective is triclabendazole, which will remove developing flukes from a few days after ingestion. Rafoxanide, nitroxynil and clorslon are effective against six week old flukes at normal dose rates and at increased dose levels affect those four weeks old. Albendazole, netobimin and oxyclozanide at normal dose rates remove only adult flukes from the bile ducts and are in effective against immature flukes. All require withholding of meat and milk for human consumption for variable periods dependent on their pharmacokinetic (Andrew *et al.,* 2003).

2.11. Control

Control of fasciolosis may be approached in two ways: by reducing populations of the intermediate snail host or by using anthelmintics.

2.11.1. Reduction of Snail Populations

Before any scheme of snail control is undertaken a survey of the area for snail habitats should be made to determine whether these are localized or widespread. The best long term method of reducing mud-snail populations such as *L.truncatala* is drainage, since it ensures permanent destruction of snail habitats. When the snail habitat is limited a simple method control is fence off this area or treats annually with a molluscicide. Currently copper sulphate is most widely used and although more efficient molluscicides, such as N-tritylmorpholine, have been developed, none are generally available. In Europe experimental evidence indicates that a mulluscicide should be applied either in the spring (May), to kill snails population prior to the commencement of the breeding, or in summer (July/August) to kill infected snails. The application a molluscicide should be combined with anthelmintic treatment to remove existing fluke populations and thus preventing contamination of habitats with eggs. When the intermediate snail host is aquatic, such as *L.tomentosa*, good control is possible by adding a molluscicide, such as N-trityl morpholine, to the water habitat of the snail, but there are many environmental objections to the use of molluscicides in water or irrigation channels (Urquhart *et al.*, 1996).

2.11.2. Use of Anthelmintics

The prophylactic use of fluke anthelmintics is aimed at: Reduction of pasture contamination by fluke eggs at a time most suitable for their development, i.e. April to August. Removing fluke populations at a time of heavy burdens or at a period of nutritional and pregnancy stress to animal. To achieve these objectives, the following control program for sheep in the Britain is recommended for year with normal or below average rain fall. Since the time of treatment is based on the fact that most metacercaria appear in autumn and early winter, it may require modification for the use in other areas. In the late April, early May treat all adult sheep with a drug effective against adult and immature stages. At this time, products containing both fasciolocide and a drug effective against nematodes which contribute to the per-parturient rise in fecal egg count in ewes may be used. Prophylactic treatment in cattle is therefore directed at reducing the flock burden in winter at a time when the parasites are susceptible to available drugs and when the nutritional status of the animal is at its lowest. It is unlikely that their faeces will remain free from eggs for any length of time as the immature stages not removed by the anthelmintic will soon develop to the adult stage. In the British Isles it is usual to treat cattle in fluke areas in mid-winter (Urquhart *et al*., 1996).

2.12. Public Health Importance

Human acquire infection through ingestion of metacercaria that are attached to certain aquatic plant, such as watercress with encysted metacercariae.and vegetable (Mas coma *et al.*, 1999; Taylor*et al*., 2007). In addition experimental studies suggested that human consuming raw liver dish from liver infected with juvenile flukes could become infected (Taylor*et al*., 2007).

Surveys in several regions indicate that there are areas with true endemic human Fasciolosis, ranging from low to very high prevalence and intensity. Recent estimates suggest that up to 2.4 million or even up to 17 million people are infected with *F. hepatica* in the world. (WHO, 2008).

Many infected persons are asymptomatic during the migration of the larvae, though some experience fever and pain in the right upper quadrant of the abdomen with an associated eosinophilia and general malaise of varying degree, including myalgia and urticarial (Boray *et al.*, 2003).

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted in Sendafa-Beke municipal abattoir of Oromia Special zone surrounding Finfinne, Ethiopia. The study zone is located between geographically coordinates 9º9`N latitudes to 39° 2`E longitudes. Its altitude is 1,496 to 2,656 masl. Sendafa is about 39km north of Addis Ababa. Concerning weather, Sendafa has a moderate temperature: June, July and August are principal rainy season. Its mean annual temperature is 15.7^0c and mean annual rainfall is 1055 mm (MUDC, 2012).

3.2. Study Animals

The study was conducted on cattle slaughtered at Sendafa-Beke manucipal abattoir. The cattle slaughtered in the abattoir were bought from different local cattle markets. Some animals come directly to the abattoir from grazing while others pass through feedlots where they are routinely de-wormed and fed straw/hay based concentrate. All cattle included in the study were male and local indigenous cattle. Sometimes it is difficult to trace the origin of the animals as they usually pass a chain of markets. Age of the animal was classified as adult and old. Those animals below 7 years old were categorized as adult while those animals above 7 years were classified as old (Delahunta and Habel, 1986). Body condition was classified as poor, medium and good based on the standard described by Nicholson and Butterworth (1986).

3.3. Sampling Method and Sample Size Determination

The animals were selected by using simple random sampling method. To determine the sample size, an expected prevalence of 50% was taken into consideration since there was no research work on fasciolosis in the area. The desired sample size for the study was calculated using the formula given by Thursfield (2007) with 95% confidence interval and 5% absolute precision.

$$n = \frac{1.96^2 \times Pexp\ (1-Pexp)}{d^2}$$

Where: n = required sample size;

Pexp = expected prevalence (P=50%)

d = desired absolute precision.

Z = 1.96 for 95% confidence interval.

$n = 1.96^2\ 0.5(1-0.5)/0.05^2 = 3.84 \times 0.25/0.0025 = 384$

A total of 384 cattle were randomly selected for the study.

3.4. Study Design*:*

A cross-sectional study was conducted from November, 2014 to April, 2015 to investigate the prevalence of bovine fasciolosis in slaughtered cattle at Sendafa-Beke manucipal abattoir. In addition, primary data was also collected on body condition and age of the animals by physical examination of animals before slaughter at the abattoir. Origin of the animals were obtained by asking the owners and recorded if known. The diagnostic sensitivity and specificity of the sedimentation technique was determined in comparison with the post-mortem findings. Fecal samples were collected during ante-mortem examination and the animals were given identification number for the subsequent postmortem examination of liver.

3.4.1. Fecal Sample Collection and Examination

Fecal samples were collected directly from the rectum of the animals using sterile disposable plastic gloves. The samples was taken to the laboratory in tightly closed universal bottles and examined for *Fasciola* eggs using sedimentation method. Each sample was serially numbered and recorded with the animals' age, sex, origin and body condition. To differentiate between eggs of paramphistome spp and fasciola spp, a drop of methylene blue solution (1%) was added to the sediment. Eggs of fasciola spp showed yellowish color while egg of paramphistome spp stained by methylene blue and the granule are transparent (Hansen and Perry, 1994, Urquhart *et al*., 1996).

3.4.2. Post-Mortem Examination

During meat inspection, livers of previously identified animals were carefully examined for the presence of *Fasciola* species. Liver inspection was carried out by visual examination and incision of the organs (FAO, 2003). For Identification of *Fasciola* species involved was carried out based on the morphology and size parameters (Soulsby, 1982; Urquhart et al., 1996).

4. DATA MANAGEMENT AND ANALYSIS

The data collected during the coprological and post mortem findings were coded and entered in Ms-Excel spreadsheet for statistical analysis. The data was analyzed using SPSS version 20.0 software. The prevalence of fasciolosis was calculated by dividing the number of cattle harboring *Fasciola* parasites by the number of cattle examined. Pearson's chi-square (X^2) was used to measure association between prevalence of the parasite with the potential risk factors. To determine the presence of significant association a p-value ≤ 0.05 was considered at 95% confidence level. Two by two table was used to compare the sedimentation and post-mortem findings.

5. RESULTS

5.1. Prevalence and Species of Fasciola Identified up on Postmortem Examination

Out the total of 384 cattle examined, 134 (34.89 %) revealed the presence of *Fasciola* species on post-mortem examination. The prevalence was 21.6% (N=83) upon coprological examination for fasciolosis. The highest prevalence of fasciolosis was observed in poor body condition cattle when compared to cattle with medium and good body conditions and the difference was statistically significant ($P < 0.05$), but statistical significant difference was not observed ($P>0.05$) among origins and age groups of the animals as shown in Table 1.

Table 1: Prevalence of bovine fasciolosis based on body condition, age and origin

Category	**Variable**	**No examined**	**Positive**	**Prevalence (%)**	**X^2**	**P-value**
BCS	Poor	44	29	65.9%	21.715	.000
	Medium	222	72	32.4%		
	Good	118	33	28.0%		
Age	Adult	309	107	34.6%	.05	.823
	Old	75	27	36.0%		
Origin	Bereh	148	53	35.8%	1.107	.953
	Aleltu	65	25	38.5%		
	Qinbibit	44	16	36.4%		
	Gimbichu	32	10	31.2%		
	Kara	19	6	31.6%		

BCS = Body conditions

Of the total 134 positive samples on post-mortem examination, *Fasciola hepatica* was the most common with 55.97% (N=75) occurrence followed by *F. gigantica* (17.91%, N=24). Mixed infection with the two species and immature flukes were also observed as indicated in table 2

Table 2: proportion of *Fasciola* species found in infected liver

Species of *Fasciola*	**No of liver infected**	**Percentage (%)**
F. hepatica	75	55.97%
F. gigantic	24	17.91%
Mixed infection	12	8.96%
Unidentified (immature)	23	17.16%
Total	134	100%

5.2. Comparison of Coprological and Post Mortem Examination

From the total 384 cattle examined for the presence of *Fasciola*, post mortem finding revealed better result (34.89%) than coprological examionation (21.6%), which showed us that the sedimentation technique used for *Fasciola* egg assessment was failed to detect eggs from some faecal samples (Table 3).

Table 3: Comparison of coprological and post mortem examination

		Post mortem examination		**Total**
		Positive	Negative	
Coprological examination	Positive	83	0	83
	Negative	51	250	301
Total		134	250	384

Sensitivity=83/134= 61.9%

Specificity= 250/250=100%

6. DISCUSSION

The overall prevalence, about 35%, of bovine fasciolosis observed by post mortem examination in this study is in agreement with the report of Mulugeta, *et al.*, (2011) from Asella abattoir who reported 34.97% prevalence. However, it is lower when compared to the result of other workers from different parts of the country such as Yilma and Mesfin (2000) who reported a 90.7% prevalence of fasciolosis in cattle slaughtered at Gondar abattoir. Tolosa and Tigre (2007) also recorded a prevalence of 46.2% at Jimma abattoir and Demissie *et al*., (2012) reported 54.5% prevalence of fasciolosis from the same abattoir. However, it is higher than 25.33% prevalence reported by Wondosen *et al*., (2012) from Wolaita Sodo municipal abattoir, 29.75% report by Nega *et al*., (2012) at Gondar ELFORA abattoir and 20.3% report by Aragaw *et al*., (2012) at Addis Ababa abattoir.

One of the most important factors that influence the occurrence of fasciolosis in an area is availability of the suitable snail habitat. Optimal temperature to the levels of 10 ºC and 16ºC are necessary for snail vectors of *Fasciola hepatica* and *Fasciola gigantica*, respectively (Soulsby, 1982; Urquhart et al., 1996). Difference in prevalence among the geographical locations therefore, is attributed mainly to the variation in the climatic and ecological conditions such as altitude, rainfall and temperature from where the animals were brought to the abattoir. Management practice and suitability of the environment for survival and distribution of the parasite may have played a role in such differences.

The current study indicated that animals with good body condition had less prevalence of faciola infection. This could be due to a better immunity in animals with good body conditions. It may also signify the importance of fasciolosis in causing weight loss, a characteristic sign of the disease (Graber, 1978, Truncy, 1989, Urquhart *et al*., 1996, Radostitis *et al*., 2007).

Statistical analysis of infection rates on the basis of age indicated a fairly similar prevalence, about 35% in adult and 36.0% in old animals. This showed that age had no effect for the occurrence of fasciolosis. This might be due to grazing of all age groups in similar fasciola contaminated pasture land (Tayler *et al*., 2007). Similar results which support the present finding were reported by Mitiku (2011) that indicated 30.2% in adults and 34.9% in olds, as well as Yasin (2012) also reported

31.10% in adults and 37.14% in olds. In contrary to these, results indicating inverse association of prevalence rate and age of cattle were reported by Mulugeta, *et al* (2011); 61.78% in adults and 20.0% in olds.

This study also revealed that there is no significant difference (P>0.05) among the different origins of the animals with respect to the prevalence of Fasciolosis. This could be attributed to similarity of the agro-ecological conditions such as altitude, rainfall and temperature favouring the development of intermediate hosts and the parasite stages as all the origins are in the near vicinity of the abattoir.

Fasciola species identification in this study revealed *F. hepatica* to be the most prevalent (55.97%) compared to *F. gigantica* (17.91%), mixed infection (8.96%), and unidentified species (17.16%). Similar findings were reported by Sisay and Nibret (2013) who reported (69.5%), (14.4%), (6.9%) and (9.2%) of *F. hepatica*, *F. gigantica*, mixed and unidentified (immature) respectively. It is also in agreement with the reports of Nega *et al.*, (2012), Aragaw *et al*., (2012), Mulgeta, *et al*., (2011), Aregay *et al*., (2013), Bekela *et al.*, (2010), Abebe *et al*., (2010), Belay,*et al.*, (2012), Tolosa and Tigre (2007); Berhe *et al*., (2009); and Ibrahim *et al*., (2010). However, in another study, Abunna *et al.*, (2009) stated that the most common liver fluke species affecting cattle at Wolaita Soddo was *Fasciola gigantica*, and studies in other countries of Africa, reported *F. gigantica* as a predominant species encountered in bovine and ovine (Phiri *et al*., 2006; Yabe *et al*., 2008). The prevalence of fasciolosis and the occurrence of a specific type of Fasciola species are known to vary with locality. The highest prevalence of *F. hepatica* in the current study might be associated with the existence of favorable ecological biotopes for the snail, *Lymnaea truncatula*, in Ethiopia (Graber and Daynes, 1974). It has been reported that there exists variation in the degree of *F. hepatica* occurrence in all areas of the country except in the arid north-east and east of the country. *F. gigantica* has been reported to occur in the western zone of the country with localized foci in the south and east. *Fasciola gigantica* in Ethiopia is found at altitudes below 1800 meters above sea level. While *Fasciola hepatica* is found at altitude of 1200- 2560 meters above sea level. Mixed infections by both species can be encountered at 1200-1800 masl (Yilma and Malone, 1998).

The prevalence of fasciolosis found in the present study was higher by post mortem finding than the coprological examination. This finding was in line with that of Nega *et al.,* (2012) and Wondosen *et al*., (2012) who reported that the post mortem prevalence was higher than that of coprological examination, this may be due to the need of longer period from 8-15 weeks after infection for the appearance of *Fasciola* egg in the feces (Radiostatis *et al*., 2007). Coprological examination includes numerous steps that increase the chance of losing eggs, as demonstrated by the lower number of positive result recorded in this work. Eggs may remain in the debris while filtering the feces through gauze or may get fixed on the bottom and wall of the container and within the pipette when taking the sediment for microscopic observation. Furthermore the detection of *Fasciola* eggs and the appearance of the disease in some areas were difficult to detect during the prepatent period because eggs are expelled intermittently depending on the evacuation of the gall bladder and life cycle of *Fasicola (*Truncy, 1989).

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, the present study confirmed that fasciolosis is highly prevalent in cattle slaughtered at Sendafa-Beke municipal abattoir. As cattle slaughtered at Sendafa-Beke municipal abattoir are bought from different cattle markets inthe nearby districts, it can be concluded that fasciolosis is still prevalent in cattle in Sendafa-Beke and its surrounding woredas. The high level of *Fasciola* in cattle in the present study represent high rate of infection and immense economic losses to the country. It was also observed that coprological examination for the parasite eggs has significant limitations in detecting the presence of fasciolosis in animals. Therefore, based on the current findings the following recommendations are forwarded:

- Strategic control of liver fluke in the study area is recommended to reduce the burden of fasciolosis and the subsequent economic loss.
- In the diagnosis of faciolosis, epidemiological information and clinical manifestations should be considered in negative fecal sample results.
- Animal owners should be aware of the effect of fasiolosis on livestock and proper implementation of control and prevention methods to reduce the burden
- Further epidemiological investigations should be initiated to assess the worm burden in Ethiopia, study the associated risk factors and economic losses.

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8. ANNEXES

Annex 1: Age determination based on dental eruption

Age/year	Characteristic
1.5-2	I_1 erupted
2-2.5	I_2 erupted
3	I_3 erupted
3.5-4	I_4 erupted
5	all incisors in wear
6	I_1 is leveled and neck has emerged from gum
7	I_2 is leveled and neck has emerged from gum
8	I_3 is leveled and neck has emerged from gum
9	I_4 is leveled and neck has emerged from gum
10	dental stars is squared in I_1 and in all teeth by 12 years
15	the teeth that are not fallen out are reduced

Note: I= Incisor

Sources: (Delahunta and Habel, 1986)

Adult < or =7 years, Old >7 years

Annex 2: Data collection sheet formed during the study period

ID.No	Origin	BCS (P, M, G)	Age (A, O)	Sex (M, F)	Fecal Result (+, -)	PM-result (+, -)	Fasciola spp			
							hep	gig	Mix	imm
1										
2										
3										
4										

Annex 3: Body condition score determination

Score	Features
1	Marked emaciation (animals would be condemned at ante mortem examination)
2	Transverse process project prominently
3	Individual dorsal spines are pointed to touch, hips, tail head, and rips are prominent transverse process visible usually individual
4	Ribs, hips and spine clearly visible muscle mass b/n hooks and spines slightly concave, slightly more flesh above the transverse process
5	Ribs usually visible, little fat cover, dorsal spines barely visible
6	Animals smooth and well covered, dorsal spines cannot be seen, but easily felt
7	Animals smooth and well covered, but fat deposit are no marked, dorsal spines can be felt with firm pressure, but rounded rather than sharp
8	Fat cover in critical areas can be easily seen and felt, transverse process cannot be seen
9	Heavily deposits of fat clearly visible on tail head, brisket, dorsal spines, ribs, hooks and spine fully covered and cannot be felt even with firm pressure

NB: 2, 3- are poor 4, 5, 6 are medium 7, 8, 9 are good.

No.1 is not slaughtered in the abattoir during my study period.

Source: (Nicholson and Butterworth, 1986)

Annex 4: procedures for sedimentation technique

Procedure:

1. Weigh or measure approximately 3 gram of faces in to container
2. Pour 40-50 ml of tap water in to container
3. Mix or stir thoroughly with a stirring device (fork, tongue blade).
4. Filter the fecal suspension through a tea strainer or double layer of cheesecloth in to another (container 2)
5. Pour the filtered material into a test tube
6. Allow standing (sediment) for 5 minutes or centrifuge 1500rpm for two minutes if the material available
7. Remove (pipette, decant) the supernatant very carefully
8. Resuspend the sediment in 5 ml of water
9. Allow the sediment for 5 minutes
10. Discard (pipette, decant) the supernatant very carefully
11. Stain the sediment by adding one drop of methylene blue
12. Transfer the sediment to a slide by pipette
13. Cover with a cover slip
14. Examine under a microscope at 4x, 10x or 40x objectives magnification

Source: (Hansen and Perry, 1994)

9. DECLARATION

I, the under signed, declare that the information presented here in my thesis is my original work, has not been presented for degree in any other university and that all sources of materials used for the thesis have been duly acknowledged

Name: Taye Degu

Signature:______________

Date of submission:________________

This thesis has been submitted for examination with my approval as university advisor

Name: Dr. Reta Tesfaye (DVM, MSc, Assistant professor)

Signature:_______________